REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	12/12/00	Final 01Aug98-30Jun99

4. TITLE AND SUBTITLE

In Vivo Incorporation of Unnatural Amino Acids Into Proteins

5. FUNDING NUMBERS

[redacted]
DAAG55-98-1-0467

6. AUTHOR(S)

Dr. Peter G. Schultz

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

[redacted] UC BERKELEY
BERKELEY CA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U. S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

ARO 37566.1-LS

11. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12 a. DISTRIBUTION / AVAILABILITY STATEMENT	12 b. DISTRIBUTION CODE
Approved for public release; distribution unlimited.	

13. ABSTRACT (Maximum 200 words)

A new orthogonal suppressor tRNA was derived from $tRNA_2^{Gln}$, which is not a substrate for any *E. coli* aminoacyl-tRNA synthetase, yet functions with the *E. coli* translational machinery. Importantly, *S. cerevisiae E. coli* glutaminyl-tRNA synthetase (GlnRS) aminioacylates the yeast orthogonal tRNA *in vitro* and in *E. coli*, but does not charge *E. coli* tRNA. This suppressor tRNA and yeast GlnRS thus represent a completely orthogonal pair in *E. coli* suitable for the delivery of unnatural amino acids into proteins *in vivo*. A general method was developed to select for mutant synthetases capable of charging any ribosomally-accepted molecule onto an orthogonal suppressor tRNA. Finally, a rapid nonradioactive screen for unnatural amino acid uptake was developed and applied to a collection of 138 amino acids. Taken together, these steps clear the way for the final phase of our efforts. Selections for mutant yeast GlnRS enzymes that accept unnatural amino acids will be undertaken. These include: (1) a two-step selection with a positive selection based on suppression of b-lactamase in the presence of unnatural amino acids, and a negative barnase selection in the absence of amino acid; (2) a screen based on recognition of a suppressed OmpA epitope; and (3) a screen based on suppression of GFP.

14. SUBJECT TERMS	15. NUMBER OF PAGES
	2
	16. PRICE CODE

17. SECURITY CLASSIFICATION OR REPORT	18. SECURITY CLASSIFICATION ON THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)
Prescribed by ANSI Std. 239-18
298-102

We have completed several key steps toward a general method to allow the site-specific incorporation of unnatural amino acids into proteins *in vivo*. Our approach involved the generation of an "orthogonal" suppressor tRNA that is uniquely acylated in *E. coli* by an engineered aminoacyl-tRNA synthetase with the desired unnatural amino acid. To this end, eight mutations were introduced into *E.coli* $tRNA_2^{Gln}$ based on an analysis of the X-ray crystal structure of the GlnRS- $tRNA_2^{Gln}$ complex and on previous biochemical data. The resulting tRNA satisfies the minimal requirements for the delivery of an unnatural amino acid: it is not acylated by any endogenous *E. coli* aminoacyl-tRNA synthetase including GlnRS, and it functions efficiently in protein translation. Repeated rounds of DNA shuffling and oligonucleotide-directed mutagenesis followed by genetic selection resulted in mutant GlnRS enzymes that efficiently acylate the engineered tRNA with glutamine *in vitro*. The mutant GlnRS and engineered tRNA also constitute a functional synthetase-tRNA pair *in vivo*. The nature of the GlnRS mutations, which occur both at the protein-tRNA interface and at sites further away, is discussed.

A new "orthogonal" suppressor tRNA was derived from *S. cerevisiae* $tRNA_2^{Gln}$. This yeast orthogonal tRNA is not a substrate *in vitro* or *in vivo* for any *E. coli* aminoacyl-tRNA synthetase, including *E. coli* glutaminyl-tRNA synthetase (GlnRS), yet functions with the *E.coli* translational machinery. Importantly, *S. cerevisiae* GlnRS aminoacylates the yeast orthogonal tRNA *in vitro* and in *E. coli*, but does not charge *E. coli* $tRNA_2^{Gln}$. This yeast-derived suppressor tRNA together with yeast GlnRS thus represents a completely orthogonal tRNA/synthetase pair in *E. coli* suitable for the delivery of unnatural amino acids into proteins *in vivo*. A general method was developed to select for mutant aminoacyl-tRNA synthetases capable of charging any ribosomally accepted molecule onto an orthogonal suppressor tRNA. Finally, a rapid nonradioactive screen for unnatural amino acid uptake was developed and applied to a collection of 138 amino acids. The majority of glutamine and glutamic acid analogs under examination were found to be uptaken by *E. coli*.

Large libraries of mutant yeast GlnRS enzymes were generated by DNA shuffling and subjected to selections for acceptance of ketone-containing amino acids and to general selections for acceptance of any unnatural amino acid. Unnatural amino acids were assigned to groups to maximize cellular uptake. Coverage of yeast GlnRS libraries was directed towards residues that were suspected of determining amino acid specificity based on the *E. coli* GlnRS structure. Positive and negative selections conditions are being explored to minimize background while preserving maximal sensitivity.